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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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LA HAYE, LE





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Titre de l'invention

Proteins Capable of Triggering G2/M transition and of Interacting with CDC2- and Cyclin B

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Remarks
Remarques

The original title of the application reads as follows:

Protein with cell proliferation and cell division promoting activity
and DNA encoding such a protein.



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Exemplar 1 DESC

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Protein with cell proliferation and cell division promoting activity and DNA
encoding such protein

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Protein with cell proliferation and cell division promoting activity and DNA encoding such protein

Specification

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The present invention relates to DNA sequences, expression vectors containing such DNA sequences, proteins encoded thereby, the use of these proteins for inducing oocyte maturation or promoting cell division and
10 in a pharmaceutical composition, uses as diagnostic markers or for identifying substances blocking the cell cycle progression and/or cell proliferation and/or differentiation, as well as further applications derived therefrom.

15 Proteins influencing cell division, proliferation or differentiation are generally of great interest. These substances open up a variety of possible uses which can be of interest for several applications depending on the specificity of these proteins. Usually, drugs which make use of the effect of suitable proteins to control or prevent pathological situations can also be
20 derived therefrom. In general, newly found proteins which can be produced recombinantly are therefore received with great interest. They do not only have potential pharmaceutical effects themselves but can often also be used as diagnostic means or as means for developing secondary pharmaceutical agents.

25

With the current systematical elucidation of the sequences of the human genome (human genome project), many sequences are found which obviously code for proteins. In most cases, though, the function of these proteins is completely unknown, so that it cannot be foreseen which
30 possible uses such products might have.

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The object underlying the present invention was to detect sequences coding for proteins influencing the cell cycle, cell division and cell proliferation. It was a further object of the present invention to produce corresponding proteins.

5

In accordance with the present invention this object was accomplished by providing a DNA sequence characterized in that it contains:

- (a) a sequence as shown in SEQ ID NO.1 or 2,
- (b) a sequence which encodes the same protein as (a) but is degenerate as a result of the genetic code,
- (c) a sequence hybridizing under stringent conditions to the sequences of (a) and/or (b),
- (d) a genomic sequence containing the sequence of (a), (b) or (c) and further containing one or more introns,
- (e) a sequence which differs from (a), (b), (c) or (d) due to its origin from a different species.

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In order to find proteins involved in cell cycle activation, a *Xenopus* oocyte cDNA was prepared and cloned in expression vectors. The primary library was subdivided into pools and plasmid DNA was purified from the pools and in vitro transcribed to obtain mRNAs. The mRNA pools were injected into stage VI oocytes which were incubated to allow for protein expression. Pools which upon microinjection in oocytes were capable of inducing oocyte maturation on their own or of strongly accelerating progesterone-induced maturation were subdivided into smaller pools and reinjected until single positive clones were isolated. Following this approach, out of a huge number of mRNA pools two specific sequences corresponding to SEQ ID NOs.1 and 2 were isolated. These sequences do not correspond by DNA hybridization experiments to any known proteins inducing oocyte maturation, including protein kinase Mos, the protein phosphatase cdc25 and several A and B type cyclins. The mRNA prepared from the two isolated clones containing SEQ ID NO.1 or 2 was used for protein expression and

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the obtained proteins were found capable of potently inducing oocyte maturation also in the absence of progesterone stimulation. The obtained DNA sequence data as shown in SEQ ID NOs.1 and 2 demonstrate that these two clones contain open reading frames coding for related proteins.

5

The present invention comprises the sequences shown in SEQ ID NOs.1 and 2 which, however, may also contain certain deviations. In particular, the present invention covers deviations which are present in the DNA only but which, owing to the diversity of the genetic code, encode the same protein as SEQ ID NO.1 or 2. Furthermore, the present invention comprises sequences which hybridize under stringent conditions with SEQ ID NO.1 or 2, or sequences deviating therefrom as set out under (b). The present invention also comprehends the corresponding genomic sequences of the cDNA sequences of SEQ ID NO.1 or 2, or of sequences deviating therefrom as set out under (b) or (c). Such genomic sequences may contain one or more introns which are cleaved off during translation and processing and thus do not influence the finally encoded protein.

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Still further, the present invention comprises sequences which deviate from those of (a), (b), (c) or (d) owing to their origin from a different species.

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Very often, highly conserved DNAs coding for proteins which have the same activities in different species, such as mouse or human, show only slight differences. In most cases, deviations occur only in some nucleotides and/or in few amino acids of the coded protein. Hence, by means of the concretely disclosed sequences as of SEQ ID NO.1 or 2, corresponding nucleic acids in other species, which code for proteins with the same or a very similar activity, can easily be found. Such similar sequences are therefore comprised by the present invention, too.

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The DNA sequences according to the invention encode proteins capable of inducing oocyte maturation and/or promoting cell division, proliferation and/or differentiation.

5 In a preferred embodiment of the invention the DNA sequences further contain expression controlled sequences which are operably linked to the coding DNA sequence. Any suitable expression control sequences may be used for the present invention. Particularly preferred sequences are those which allow a favourable control of expression, such as sequences allowing
10 induction of expression or inhibition of expression. Induction or inhibition generally takes place via the binding of a respective inductor or inhibitor molecule to operator sequences. Corresponding expression control sequences are known to the person skilled in the art, the lac operator being an example therefor.

15 A further subject matter of the present invention is an expression vector containing a DNA sequence according to the invention.

As set out above for the DNA sequences, the expression vector also particularly preferably comprises expression control sequences allowing for
20 specific expression control. Also, sequences that allow for positive selection of transformed host cells are known to the man in the art and are preferably introduced in the expression vectors according to the invention.

25 A further subject matter of the present invention is a protein encoded by a DNA sequence according to the present invention. As explained above, the protein according to the invention, which preferably contains an amino acid sequence according to SEQ ID NO.3 or 4, induces and stimulates cell proliferation and differentiation. Oocyte maturation is also induced by the
30 proteins of the invention. The particularly preferred proteins of SEQ ID NOs.3 and 4 are capable of inducing oocyte maturation considerably faster

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than the same amount of injected malE-Mos or progesterone treatment. The entire cell cycle in *Xenopus* oocytes is extraordinarily strongly activated by the proteins of the invention. Only low amounts of the protein of the invention are required to stimulate oocyte maturation, cell proliferation and differentiation.

The protein according to the present invention may have deletions, substitutions and/or additions of amino acids in regions which do not affect the activity. However, the activity of the protein must not be considerably impaired thereby. Further, it is preferred that at the most up to 5 % of the amino acid content of the protein of the invention has deletions, substitutions and/or additions of amino acids. It is not difficult for the skilled artisan to find out which regions may contain deletions, substitutions or additions. Corresponding changes can be made in the nucleic acids, followed by expression and an activity test. By means of site-directed mutagenesis manifold variants can easily be produced and expressed. The person of skill in the art can easily simultaneously test a multitude of such mutants for their activity (high-throughput screening), whereby as a prerequisite at least half the activity of the proteins shown in SEQ ID NO.3 or 4 has to be retained. By means of computer-aided conformation studies the regions of the protein which are less probably involved in the activity of the protein can be determined. Particularly in such regions can mutations be made.

A still further subject matter of the present invention is the use of the protein according to the invention for inducing oocyte maturation and/or promoting cell division, cell proliferation or cell differentiation.

As a further subject matter of the present invention a pharmaceutical composition may be formulated on the basis of this possible use. The pharmaceutical composition according to the invention contains as active agent a protein according to the invention which in particular contains the

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amino acids as of SEQ ID NO.3 or 4 or sequences derived therefrom which may exhibit the aforementioned mutations, deletions or substitutions.

Preferably, the pharmaceutical composition contains the protein in combination with a pharmaceutically acceptable carrier or adjuvant.

The pharmaceutical composition according to the invention may be used for all pathological situations in which it is desired to promote cell proliferation, cell differentiation or cell maturation. Examples for such applications are the promotion of growth and maturation of specific cell types, e.g. ovarian cells, so that the pharmaceutical composition of the invention is in particular also useful and suitable for fertility treatments.

The protein according to the invention can further be used as diagnostic marker for cell proliferation and/or cell differentiation. The amount of said protein contained in an organism can be correlated to the cell proliferation or differentiation rate. As soon as a basic value has been determined, the amount of this protein present in, e.g., different development stages of cells can be determined, thus showing the particular development status.

A further possible use of the proteins according to the invention lies in their capability of acting as a target for the identification of drugs blocking cell cycle progression and/or cell proliferation and/or cell differentiation. By means of these proteins a multitude of substances can be tested for their blocking and inhibition capability. To this end, a system is provided which comprises cells susceptible to the proteins of the invention, proteins according to the invention and a substance which is to be examined as to its blocking activity. It can then be determined whether the activity of the protein to promote cell proliferation and/or differentiation is weakened or even prevented by said substance. Using high-throughput screening (HTPS), such experiments can be carried out for a multitude of substances simultaneously. A particularly preferable use for identifying substances

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blocking cell proliferation and/or differentiation lies in the development of drugs for the treatment of cancer or other pathological situations with uncontrolled cell proliferation.

- 5 Especially carcinoma grow by uncontrolled cell division, and the inhibition thereof is highly desired. Substances allowing to block such division can be found using the protein of the invention.

10 Another subject matter of the present invention is the use of the DNA sequences according to the invention as diagnostic marker for the cell proliferation and/or cell differentiation status, whereby the amount of homologous nucleic acids present in the cell is determined by hybridization experiments. Of particular interest is the amount of mRNAs hybridizing to the DNA according to the invention. For this purpose, preferably the DNA
15 sequence according to the invention or a part thereof is labelled, so that after performance of the hybridization experiment the formed double strands may be easily detected. Particularly preferably, a single-stranded DNA sequence should be used corresponding to the antisense strand of the DNA according to the invention. Using such an antisense strand DNA which is
20 complementary to the mRNA, the actual amount of formed protein can be determined on a nucleic acid basis.

In combination with the figures the following examples are to further illustrate the present invention.

25

Fig. 1 shows a sequence comparison of proteins Is26 and Is27.

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Fig. 2 shows the result of experiments with injection of small amounts of recombinant malE-Is26 protein (10 ng) into *Xenopus* oocytes, leading to oocyte maturation considerably faster than the same amount of injected malE-Mos or a progesterone treatment. Fig. 2 also shows the same

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experiment where cycloheximide preincubation blocked malE-Mos-induced GVBD (germinal vesicle breakdown) but had no effect on Is26-triggered oocyte maturation.

5 Fig. 3 shows the malE-Is26-induced activation of MAP kinase and cdc2/cyclin B in an immunoblot and by direct measurement of the in vitro kinase activity using MBP and histone H1 as substrates for MAP kinase and cdc2/cyclin.

10 Fig. 4 also shows an immunoblot using anti-cdc2 antibodies and an in vitro histone H1 kinase assay.

15 Fig. 5 shows a pull-down experiment using rabbit reticulocyte lysates which demonstrates that Is26 can directly bind to B-type cyclins.

Example 1

20 To identify novel proteins implicated in cell cycle activation in *Xenopus* oocytes, an expression cloning strategy was used where a *Xenopus* oocyte cDNA library was constructed in the FTX5 expression vector. The primary library was subdivided into pools of 150-200 colonies and plasmid DNA was purified from the pools and in vitro transcribed to obtain mRNAs. The
25 mRNA pools were injected into stage VI oocytes which were incubated for 30 - 36 hours to allow protein expression from the injected mRNAs prior to stimulation with progesterone. Finally, those pools which upon microinjection in oocytes were capable either of inducing oocyte maturation on their own or of strongly accelerating progesterone-induced maturation
30 were subdivided into smaller pools and reinjected until single positive clones were isolated.

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Using this approach, out of 105 mRNA pools injected in oocytes two clones were isolated which did not correspond by DNA hybridization experiments to proteins that are known to induce oocyte maturation including the protein kinase Mos, the protein phosphatase cdc25 and several A and B type cyclins. The mRNAs prepared from the two isolated clones, which were referred to as ls26 and ls27, were capable of potentially inducing oocyte maturation in the absence of progesterone stimulation. DNA sequencing showed that these two clones contained open reading frames that encode for related proteins and were fused in frame to the C-terminus of the myc tag in the FTX5 vector.

Full-length ls26 and ls27 cDNAs were cloned from a λ ZAP Xenopus oocyte cDNA library using as probes the two cDNAs isolated in the expression screening. The ls26 clone was 1574 base pairs and encoded for a protein of 300 amino acids (SEQ ID NO.1), whilst ls27 was 1357 base pairs in length and encoded for a protein of 298 amino acids (SEQ ID NO.2). Both clones contained stop codons upstream of the first ATG and in the same frame (underlined in SEQ ID NO.1 and 2). The predicted ls26 and ls27 proteins were 91% identical (Fig. 1). When the ls26 and ls27 sequences were tested against DNA and protein sequence data bases, no significant homologies (Blast search) could be detected, suggesting that ls26/ls27 belong to a novel protein family. Programmes were also tested which were designed to identify conserved protein motifs (for example Prosite), but again there was no clue as to the kind of activity that the ls26/ls27 proteins may have. Thus, based on the lack of sequence homology, the ls26/ls27 proteins do not appear to have any known catalytic activity (protein kinase, phosphatase,...).

Example 2

To investigate the function of the ls26/ls27 proteins, the two cDNAs were cloned in the bacterial expression vector pMalc2 downstream of the malE

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gene. The fusion proteins malE-Is26 and malE-Is27 were expressed in and purified from *E. coli*. Since Is26 and Is27 are very similar in sequence and probably correspond to pseudoalleles, which are quite common in *Xenopus*, one concentrated on the characterization of Is26 and then confirmed the results obtained using Is27. It was found that the injection into *Xenopus* oocytes of small amounts of recombinant malE-Is26 protein (10 ng) was capable of inducing oocyte maturation considerably faster than the same amount of injected malE-Mos or than progesterone treatment (Fig. 2). This experiment using the fusion protein confirmed the results observed with mRNA in vitro transcribed from the Is26 cDNA clone regarding the potency of this novel protein to induce cell cycle activation in *Xenopus* oocytes. It was also found that injection of only 0.5 ng of malE-Is26 per oocyte was still capable of inducing oocyte maturation. The availability of purified malE-Is26 protein also allowed to test the capability of Is26 to induce oocyte maturation in the presence of protein synthesis inhibitors. Preincubation of the oocytes with cycloheximide totally blocked progesterone-induced maturation, consistent with the known essential requirement for translation of maternal mRNAs stored in the oocytes for progesterone to induce maturation. In the same experiment, cycloheximide preincubation also blocked malE-Mos-induced GVBD but it had no effect on Is26-triggered oocyte maturation (Fig. 2).

Example 3

To further characterize the activity of the Is26 protein, the kinetics of activation of MAP kinase (MAPK) and cdc2/cyclin B (MPF) in oocytes induced to mature by malE-Is26 were investigated. MAPK and MPF are normally activated during oocyte maturation and their activation can be detected in oocyte lysates either by immunoblot with anti-MAPK and anti-cdc2 antibodies or by direct measurement of the in vitro kinase activity using MBP and histone H1 as substrates for MAPK and MPF, respectively (Fig. 3). As expected from previous work, we observed that progesterone

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treatment activates both MAPK and MPF at about the same time, whereas malE-Mos injection activates MAPK well before MPF activation. Interestingly, injection of malE-Is26 rapidly activates MPF somewhat before MAPK. Moreover, MPF appears to be transiently activated by Is26, but the
5 significance of this observation is unclear. In cycloheximide-treated oocytes, the Is26-induced activation of MAPK is very much reduced whereas the activation of MPF is apparently unaffected. This result indicates that the effect of Is26 is more related to MPF activation than to MAPK activation. As expected, cycloheximide totally blocked progesterone-induced activation
10 of both MPF and MAPK, whereas in the case of Mos only MPF but not MAPK activation was compromised by cycloheximide.

Example 4

15 The observation that Is26 can consistently induce oocyte maturation and the activation of MPF independently of new protein synthesis is quite remarkable as only proteins that act very late in the activation pathways, such as cyclins (cdc2 binding and activating subunits) or direct cdc2/cyclinB activators such as the cdc25 phosphatase have been shown to have this
20 strong effect. In order to address whether Is26 can directly associate with and/or modify the activity of cdc2/cyclin B complexes, pull-down experiments were performed. For this purpose, extracts prepared from insect cells infected with cdc2-expressing baculovirus were incubated with either malE-Is26 bound to amylose beads or the equivalent amount of cyclin
25 B bound to nickel beads. After extensive washing, the proteins that remained bound to the beads were analyzed by immunoblot using anti-cdc2 antibodies and in vitro histone H1 kinase assay. We found that Is26 bound to cdc2 with almost the same efficiency of cyclin B (Fig. 4). However, while binding of the activating cdc2 subunit cyclin B resulted in high levels of
30 histone H1 kinase activity of the cdc2/cyclin B complexed, Is26 appeared to have little or no effect to stimulate the histone H1 kinase activity of cdc2. When the cyclin B pull-down was done in the presence of a 2-fold

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molar excess of soluble malE-ls26, we observed a reduction in the amount of cdc2 bound to cyclin B which also correlated with the expected decrease in the kinase activity of the complexes. This suggests that ls26 may compete with cyclin B to bind to cdc2. By immunoblot using anti-malE
5 antibodies we confirmed that cyclin B was capable of binding ls26 in the presence of cdc2. Our results indicate that ls26 can strongly bind to cdc2 and probably also to cyclin B, but we do not know whether ls26 can complex to cdc2/cyclin B.

10 The interaction between ls26 and cdc2 was confirmed using ³⁵S-methionine-labelled cdc2 prepared by coupled transcription/translation in rabbit reticulocyte lysates. We also confirmed in pull-down experiments with rabbit reticulocyte lysates that ls26 can directly bind to B-type cyclins (Fig. 5).

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SEQ ID NO. 1

Is 26 cDNA

```
1   TTA AAC AGG ACT TGC AGC TCC AGT GTA GGT TTT TTC AGA AGC TCC GCC CCA ATG CTG TAT
61  TTT TTT ATT ATT CCA GGA GGC TAT AAA GAG AGC AGA CAA AGG AAG TAG GCG GAG TTC CTG
121 TTT ATC GCC ATT TGG CCA GTG GTG GCT AAG CGC CTG AAG GTG GCT GCT TCC TTT GCT CAG
181 ATC AAC CCT CGG GCC GGT GTC CCC CTT TCT ACA ATG AGG CAC ATG CAG AGT GTA ACC CGG
      M   R   H   M   Q   S   V   T   R   9
241 GCC AGC TCC ATT TGT GGC AGC GGG GTG AAG CAG GTC ATT GGC AAG GGG CAT CCG CAC GCC
      A   S   S   I   C   G   S   G   V   K   Q   V   I   G   K   G   H   P   H   A   29
301 CGG GTT GTT GGA GCG CGC AAG GCG CAA ATC CCT GAG AGA GAG GAG TTG TCA GTC AAA CCC
      R   V   V   G   A   R   K   A   Q   I   P   E   R   E   E   L   S   V   K   P   49
361 AAA ATG GTG CGA AAT ACC CAT CTC AAT CTA CAG CCC CAG GAG CGC CAG GCC TTC TAC AGG
      K   M   V   R   N   T   H   L   N   L   Q   P   Q   E   R   Q   A   F   Y   R   69
421 CTC CTA GAA AAT GAG CAG ATT CAG GAA TTC CTT TCT ATG GAC TCC TGT CTA AGG ATT TCC
      L   L   E   N   E   Q   I   Q   E   F   L   S   M   D   S   C   L   R   I   S   89
481 GAC AAG TAT CTC ATA GCA ATG GTT CTA GCA TAT TTT AAG CGG GCA GCG GGC CTC TAC ACC
      D   K   Y   L   I   A   M   V   L   A   Y   F   K   R   A   A   G   L   Y   T   109
541 AGC GAG TAC ACA ACC ATG AAT TTC TTT GTT GCC CTG TAT CTG GCT AAT GAC ATG GAG GAA
      S   E   Y   T   T   M   N   F   F   V   A   L   Y   L   A   N   D   M   E   E   129
601 GAT GAA GAA GAC TAT AAA TAT GAA ATC TTC CCC TGG GCA CTA GGA GAC TCG TGG CGT GAG
      D   E   E   D   Y   K   Y   E   I   F   P   W   A   L   G   D   S   W   R   E   149
661 CTT TTC CCA CAA TTT TTG CGT CTC CGG GAC GAC TTC TGG GCT AAA ATG AAC TAC CGA GCA
      L   F   P   Q   F   L   R   L   R   D   D   F   W   A   K   M   N   Y   R   A   169
721 GTT GTT AGT CGA AGG TGC TGT GAT GAG GTA ATG TCC AAA GAT CCC ACT CAT TGG GCC TGG
      V   V   S   R   R   C   C   D   E   V   M   S   K   D   P   T   H   W   A   W   189
781 CTG AGA GAT CGC CCC ATG CAT CAC AGC GGG GCC ATG CGT GGT TAC CTT AGA AAC GAG GAC
      L   R   D   R   P   M   H   S   G   A   M   R   G   Y   L   R   N   E   D   209
841 GAC TTT TTC CCC CGG GGT CCA GGC CTT ACA CCA GCC AGC TGT ACA CTT TGC CAT AAA GCA
      D   F   F   P   R   G   P   G   L   T   P   A   S   C   T   L   C   H   K   A   229
901 GGT GTC TGT GAC TCT GGT GGG GTC TCC CAC AAC AAC TCT TCC TCT CCA GAA CAA GAG ATT
      G   V   C   D   S   G   G   V   S   H   N   N   S   S   S   P   E   Q   E   I   249
961 TTT CAC TAC ACC AAT AGG GAG TGG TCC CAG GAG CTT CTC ATG TTG CCC CCT GAG CTG TTG
      F   H   Y   T   N   R   E   W   S   Q   E   L   L   M   L   P   P   E   L   L   269
1021 CTG GAT CCC GAG TGT ACT CAT GAC TTA CAC ATT CTC CAG GAG CCA TTG GTT GGA TTA GAG
      L   D   P   E   C   T   H   D   L   H   I   L   Q   E   P   L   V   G   L   E   289
1081 CCA GAT GGG ACG GCG CTG GAA TGG CAC CAC CTT TAG TAG CCG ATT GTC TCC TCC GAG CTT
      P   D   G   T   A   L   E   W   H   H   L   *   *   300
1141 TTA TTC TTC TCT ACT CAC AAG CTC AGC ACT TAT TCT CTC CTC CTA AGG ACT TGT CAA TGT
1201 TCA GAC TTA ATT GAA ATG GGA GAA GTG AAT ATT CCG ACG GAT GTA GAG CGG GAA TAT GTG
1261 CCC AGA GAA AGT GTT TTG AGT CTG TAT AAA CCG TTG CTT TGT AAA TAA ATA TAT AAA TGT
1321 TCT CTG TGC TGG TCA CTA ATA AAG ATC AGG TAA AAT CAC TTT CAG GTG TAA TTT AAT AGT
1381 ATG TAT GTA GAG TCT TTA ATT CAG CTC TCC ACC AAA TAG TAA CTT GTC ATC ACT GAA CCT
1441 TTG CTT AAC TAC ACT TTT ATT ATT CTG CAC ACA AAT ATT CTG AAG ATC AGA CCG TTC TGT
1501 TTT CAG ATG GGT TGA AAA TAT TAA ACT CAA CAG AAT TCC TGT GGT GTA ATG TAA ATG CAA
1561 AGA TCG ATT AGA CTA
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SEQ ID NO. 2

Is 27 cDNA

1	GCT	GTA	TTT	TAC	TTT	CTT	TCA	GGA	GGC	TAT	AAA	GAC	AAC	AGA	CAG	GGG	AGG	<u>TAG</u>	GCA	GAG	
61	TTC	CTG	TTC	ATC	ACC	ATT	CTT	TGG	CCG	TTG	GTG	GCT	AGG	CGC	CTG	AAG	GTG	GCT	GCT	ACC	
121	TTT	GCT	CAG	ATC	AAC	CCT	CGG	TTC	GTT	GTC	CCC	CTT	TCT	ACA	ATG	AGG	CAT	ATG	CAG	AGT	
															M	R	H	M	Q	S	6
181	GCA	ACC	CGG	GCC	ACC	TTA	GTT	TGT	GGC	AGC	GGG	GTA	AAG	CAG	ATC	ATT	GCC	AAG	GGA	CAT	
	A	T	R	A	T	L	V	C	G	S	G	V	K	Q	I	I	A	K	G	H	26
241	CCG	AAT	ACC	CGG	GTT	TTT	GGA	GCG	CGC	AAG	GCG	AAA	ATC	CCT	GAG	AGA	GAG	GTG	CTA	GCA	
	P	N	T	R	V	F	G	A	R	K	A	K	I	P	E	R	E	V	L	A	46
301	GCC	AAA	CCC	AAG	ATC	ACG	CGC	ATT	ACA	CAT	CTC	AAT	CTA	CAA	CCC	CAG	GAG	CGC	CAG	GCC	
	A	K	P	K	I	T	R	I	T	H	L	N	L	Q	P	Q	E	R	Q	A	66
361	TTT	TAC	AGG	CTC	CTA	GAA	AAT	GAG	CTG	ATT	CAG	GAA	TTT	CTT	TCT	ATG	GAC	TCC	TGT	CTA	
	F	Y	R	L	L	E	N	E	L	I	Q	E	F	L	S	M	D	S	C	L	86
421	AAG	ATT	TCA	GAC	AAG	TAT	CTC	ATA	GCA	ATG	GTT	CTA	GCA	TAT	TTT	AAG	CGG	GCG	GGC	CTC	
	K	I	S	D	K	Y	L	I	A	M	V	L	A	Y	F	K	R	A	G	L	106
481	TAC	ACC	AGC	GAG	TAC	ACA	ACC	ATG	AAT	TTC	TTT	GTT	GCT	CTG	TAT	CTG	GCT	AAT	GAC	ATG	
	Y	T	S	E	Y	T	M	N	F	F	V	A	L	Y	L	A	N	D	M		126
541	GAG	GAA	GAT	GAA	GAA	GAC	TAT	AAA	TAT	GAA	ATC	TTC	CCC	TGG	GCA	CTA	GGA	GAT	TCA	TGG	
	E	E	D	E	E	D	Y	K	Y	E	I	F	P	W	A	L	G	D	S	W	146
601	CGT	GAG	TTT	TTC	CCA	CAA	TTT	TTA	CGT	CTC	CGG	GAC	GAC	TTC	TGG	GCT	AAA	ATG	AAC	TAC	
	R	E	F	F	P	Q	F	L	R	L	R	D	D	F	W	A	K	M	N	Y	166
661	CGA	GCA	GTT	GTT	AGC	CGA	AGA	TGT	TGT	GAT	GAG	GTA	ATG	GCG	AAA	GAT	CCC	ACT	CAT	TGG	
	R	A	V	V	S	R	R	C	C	D	E	V	M	A	K	D	P	T	H	W	186
721	GCC	TGG	CTC	AGA	GAT	CGT	CCT	ATT	CAT	CAT	AGT	GGG	GCC	CTG	CGT	GGT	TAC	CTC	AGA	AAT	
	A	W	L	R	D	R	P	I	H	H	S	G	A	L	R	G	Y	L	R	N	206
781	GAG	GAT	GAC	TTT	TTC	CCT	CGG	GGT	CCA	GGC	CTT	ACA	CCA	GCC	AGC	TGT	GCA	CTT	TGC	CAT	
	E	D	D	F	F	P	R	G	P	G	L	T	P	A	S	C	A	L	C	H	226
841	AAA	GCA	AGT	GTC	TGT	GAC	TCT	GGT	GGG	GTG	TCC	CAT	GAC	AAC	TCT	TCT	CCA	GAA	CAA	GAG	
	K	A	S	V	C	D	S	G	G	V	S	H	D	N	S	S	P	E	Q	E	246
901	ATT	TTT	CAC	TAC	ACC	AAT	AGG	GAG	TGG	TCC	CAG	GAA	CTT	CTC	ATC	TTG	CCA	CCT	GAA	CTG	
	I	F	H	Y	T	N	R	E	W	S	Q	E	L	L	I	L	P	P	E	L	266
961	TTA	TTG	GAT	CCG	GAG	TCT	ACT	TAT	GAC	ATC	CAC	ATT	TTC	CAG	GAA	CCG	TTG	GTT	GGA	TTA	
	L	L	D	P	E	S	T	Y	D	I	H	I	F	Q	E	P	L	V	G	L	286
1021	GAG	CCA	GAT	GGG	GCA	GCC	TTG	GAA	TGG	CAC	CAC	CTT	TAG	CAC	CAT	GTC	ATC	TCT	GTG	CTT	
	E	P	D	G	A	A	L	E	W	H	H	L	*								298
1081	TCA	TTC	TTC	TCT	AAT	CCA	CGA	GCT	CAA	GAA	GCA	CTT	AAC	CTC	TCC	TAA	GCA	CTT	GCC	CAT	
1141	GTC	CCT	ATT	CAG	ACT	AAT	GAA	TTA	AAT	GGG	AGA	GGT	GAC	TAT	TGC	CAT	AAA	GGG	AAG	GAT	
1201	GCC	ACT	TAG	AGT	GGA	GAA	TAA	TAC	TTG	CCA	AAA	ATG	GTG	TTT	GGG	TCT	GTT	TAA	ACT	GTT	
1261	GCT	ATT	TCA	GTT	GCC	TTG	TAA	ATA	AAT	AAG	TAT	AAA	AAT	GTA	TGC	TCT	GTG	CCG	GTT	GCT	
1321	AAT	AAA	AAA	AAA	ATC	TGG	TAT	CAA	AAA	AAA	AAA	AAA	AAA	A							

SEQ ID NO.3

										M	R	H	M	Q	S	V	T	R	9	
A	S	S	I	C	G	S	G	V	K	Q	V	I	G	K	G	H	P	H	A	29
R	V	V	G	A	R	K	A	Q	I	P	E	R	E	E	L	S	V	K	P	49
K	M	V	R	N	T	H	L	N	L	Q	P	Q	E	R	Q	A	F	Y	R	69
L	L	E	N	E	Q	I	Q	E	F	L	S	M	D	S	C	L	R	I	S	89
D	K	Y	L	I	A	M	V	L	A	Y	F	K	R	A	A	G	L	Y	T	109
S	E	Y	T	T	M	N	F	F	V	A	L	Y	L	A	N	D	M	E	E	129
D	E	E	D	Y	K	Y	E	I	F	P	W	A	L	G	D	S	W	R	E	149
L	F	P	Q	F	L	R	L	R	D	D	F	W	A	K	M	N	Y	R	A	169
V	V	S	R	R	C	C	D	E	V	M	S	K	D	P	T	H	W	A	W	189
L	R	D	R	P	M	H	H	S	G	A	M	R	G	Y	L	R	N	E	D	209
D	F	F	P	R	G	P	G	L	T	P	A	S	C	T	L	C	H	K	A	229
G	V	C	D	S	G	G	V	S	H	N	N	S	S	S	P	E	Q	E	I	249
F	H	Y	T	N	R	E	W	S	Q	E	L	L	M	L	P	P	E	L	L	269
L	D	P	E	C	T	H	D	L	H	I	L	Q	E	P	L	V	G	L	E	289
P	D	G	T	A	L	E	W	H	H	L	*	*								300

SEQ ID NO. 4

														M	R	H	M	Q	S	6
A	T	R	A	T	L	V	C	G	S	G	V	K	Q	I	I	A	K	G	H	26
P	N	T	R	V	F	G	A	R	K	A	K	I	P	E	R	E	V	L	A	46
A	K	P	K	I	T	R	I	T	H	L	N	L	Q	P	Q	E	R	Q	A	66
F	Y	R	L	L	E	N	E	L	I	Q	E	F	L	S	M	D	S	C	L	86
K	I	S	D	K	Y	L	I	A	M	V	L	A	Y	F	K	R	A	G	L	106
Y	T	S	E	Y	T	T	M	N	F	F	V	A	L	Y	L	A	N	D	M	126
E	E	D	E	E	D	Y	K	Y	E	I	F	P	W	A	L	G	D	S	W	146
R	E	F	F	P	Q	F	L	R	L	R	D	D	F	W	A	K	M	N	Y	166
R	A	V	V	S	R	R	C	C	D	E	V	M	A	K	D	P	T	H	W	186
A	W	L	R	D	R	P	I	H	H	S	G	A	L	R	G	Y	L	R	N	206
E	D	D	F	F	P	R	G	P	G	L	T	P	A	S	C	A	L	C	H	226
K	A	S	V	C	D	S	G	G	V	S	H	D	N	S	S	P	E	Q	E	246
I	F	H	Y	T	N	R	E	W	S	Q	E	L	L	I	L	P	P	E	L	266
L	L	D	P	E	S	T	Y	D	I	H	I	F	Q	E	P	L	V	G	L	286
E	P	D	G	A	A	L	E	W	H	H	L	*								298



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Claims

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1. A DNA sequence,
5 characterized in that it contains:
- (a) a sequence as shown in SEQ ID NO.1 or 2,
 - (b) a sequence which encodes the same protein as (a) but is degenerate as a result of the genetic code,
 - (c) a sequence hybridizing under stringent conditions to the
10 sequences of (a) and/or (b),
 - (d) a genomic sequence containing the sequence of (a), (b) or (c) and further containing one or more introns,
 - (e) a sequence which differs from (a), (b), (c) or (d) due to its origin from a different species.
- 15
2. A DNA sequence according to claim 1,
wherein it encodes a protein that is capable of inducing oocyte maturation and/or promoting cell division.
- 20
3. A DNA sequence according to claim 1 or 2,
characterized in that it further contains expression control sequences operably linked to the coding DNA sequence.
- 25
4. Expression vector,
characterized in that it contains a DNA sequence according to anyone of claims 1 to 3.
- 30
5. Protein
characterized in that it is encoded by a DNA sequence according to anyone of claims 1 to 3.
6. Protein according to claim 5,

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characterized in that it contains an amino acid as shown in SEQ ID NO.3 or 4.

7. Protein according to claim 5 or 6,
5 characterized in that it shows an oocyte maturation inducing activity and/or a cell division promoting activity.
8. Protein according to anyone of claims 5 to 7,
10 characterized in that it contains deletions, substitutions and/or additions of amino acids that do not substantially affect its activity.
9. Protein according to anyone of claims 5 to 8,
wherein a second protein is fused to build a fusion protein.
- 15 10. Use of a protein according to anyone of claims 5 to 9 for inducing oocyte maturation and/or promoting cell division and/or differentiation and/or proliferation.
- 20 11. Pharmaceutical composition containing as active agent a protein according to anyone of claims 5 to 9.
12. Pharmaceutical composition according to claim 11, containing the protein in combination with a pharmaceutically acceptable carrier or adjuvant.
- 25 13. Use of a pharmaceutical composition according to claim 10 or 11 for promoting cell proliferation, cell differentiation, or for fertility treatments.
- 30 14. Use of a protein according to anyone of claims 5 to 9 as a diagnostic marker for cell proliferation and/or cell differentiation.

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15. Use of a protein according to claims 5 to 9 as a target for the identification of drugs that block cell cycle progression and/or cell proliferation and/or cell differentiation.

5 16. Use according to claim 15 for the development of pharmaceuticals for the treatment of cancer or other pathological situations with uncontrolled cell proliferation.

10 17. Use of a DNA sequence according to anyone of claims 1 to 3 or a part thereof as diagnostic marker for cell proliferation and/or cell differentiation for hybridization experiments to determine the amount of homologous nucleic acid sequences.

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FIGURE 1EPO - Munich
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Is 26 1 MRHMQSVTRASSICGSGVKQVIGKGHPHARVVGARKAQIPEREE
Is 27 1 MRHMQSATRATLVCGSGVKQILAKGHPNTRVFGARKAKIPEREV

Is 26 45 LSVKPKMVRNTHLNLOPQERQAEYRLLENEQIQEFLSMDSCLRI
Is 27 45 LAAKPKITRITHLNLOPQERQAEYRLLENELIQEFLSMDSCLKI

Is 26 89 SDKYLIAMVLAYFKRAAGLYTSEYTTMNEFFVALYLANDMEEDEE
Is 27 89 SDKYLIAMVLAYFKRA-GLYTSEYTTMNEFFVALYLANDMEEDEE

Is 26 133 DYKYEIFPWALGDSWRELFPOFLRLRDDEFWAKMNYRAVVSRRCC
Is 27 132 DYKYEIFPWALGDSWREFFPOFLRLRDDEFWAKMNYRAVVSRRCC

Is 26 177 DEVMSKDPTHWAWLRDRPMHHSGAMRGYLRNEDDDFFPRGPGLTP
Is 27 176 DEVMAKDPTHWAWLRDRPIHHSGALRGYLRNEDDDFFPRGPGLTP

Is 26 221 ASCTLCHKAGVCDSGGVSHNNSSSPEQEIFHYTNREWSQELLML
Is 27 220 ASCALCHKASVCDSGGVSHDNSS-PEQEIFHYTNREWSQELLIL

Is 26 265 PPELLLDPECTHDLHILOEPLVGLLEPDGTALEWHHL
Is 27 263 PPELLLDPESTYDIHIFQEPLVGLLEPDGAALEWHHL

FIGURE 2

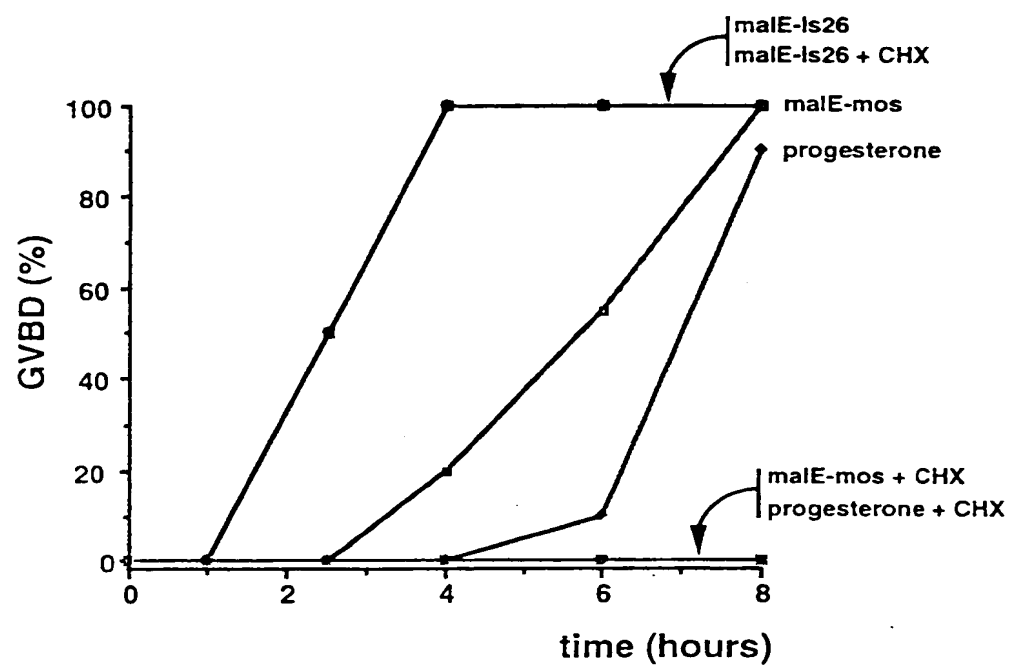


FIGURE 3

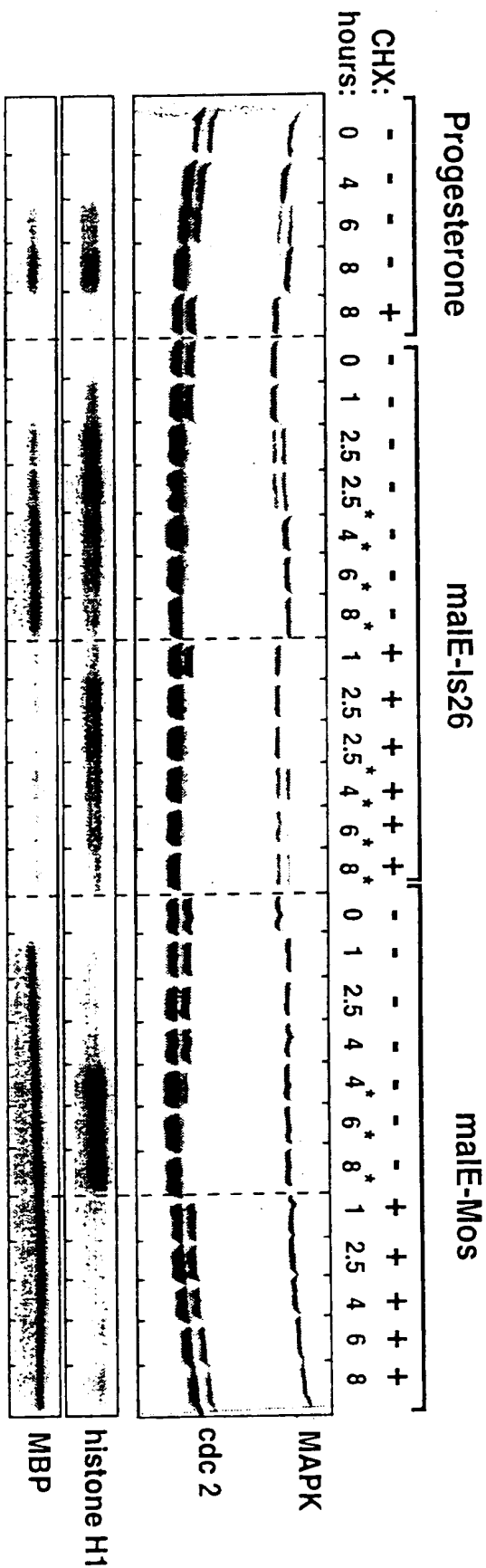
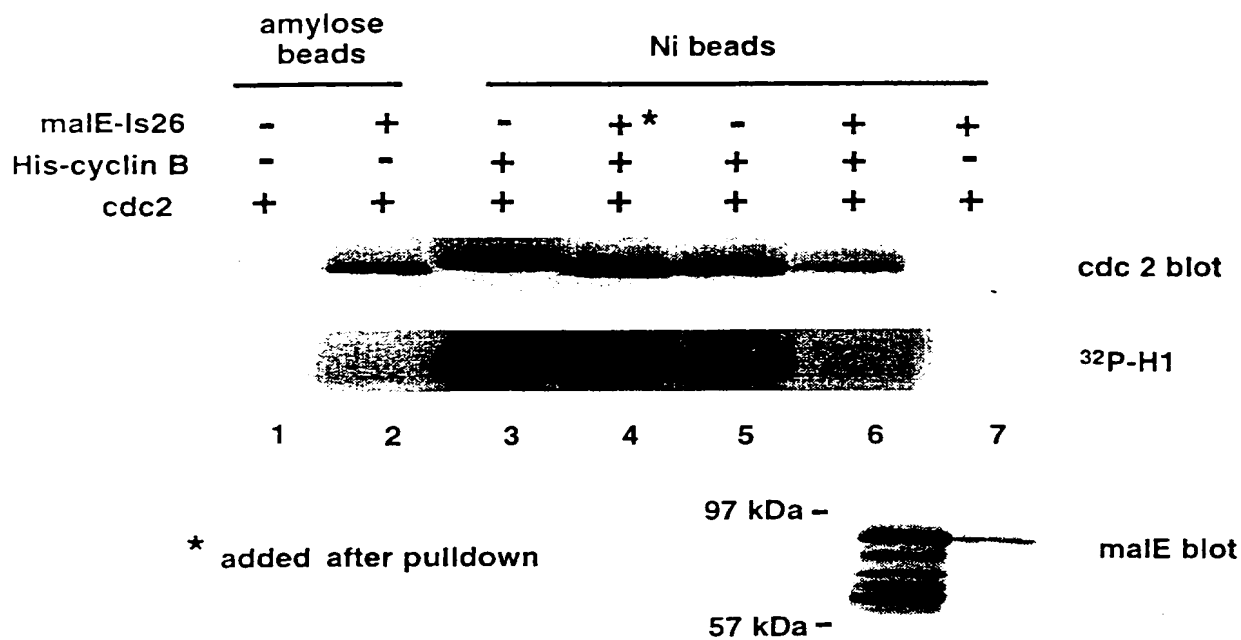


FIGURE 4



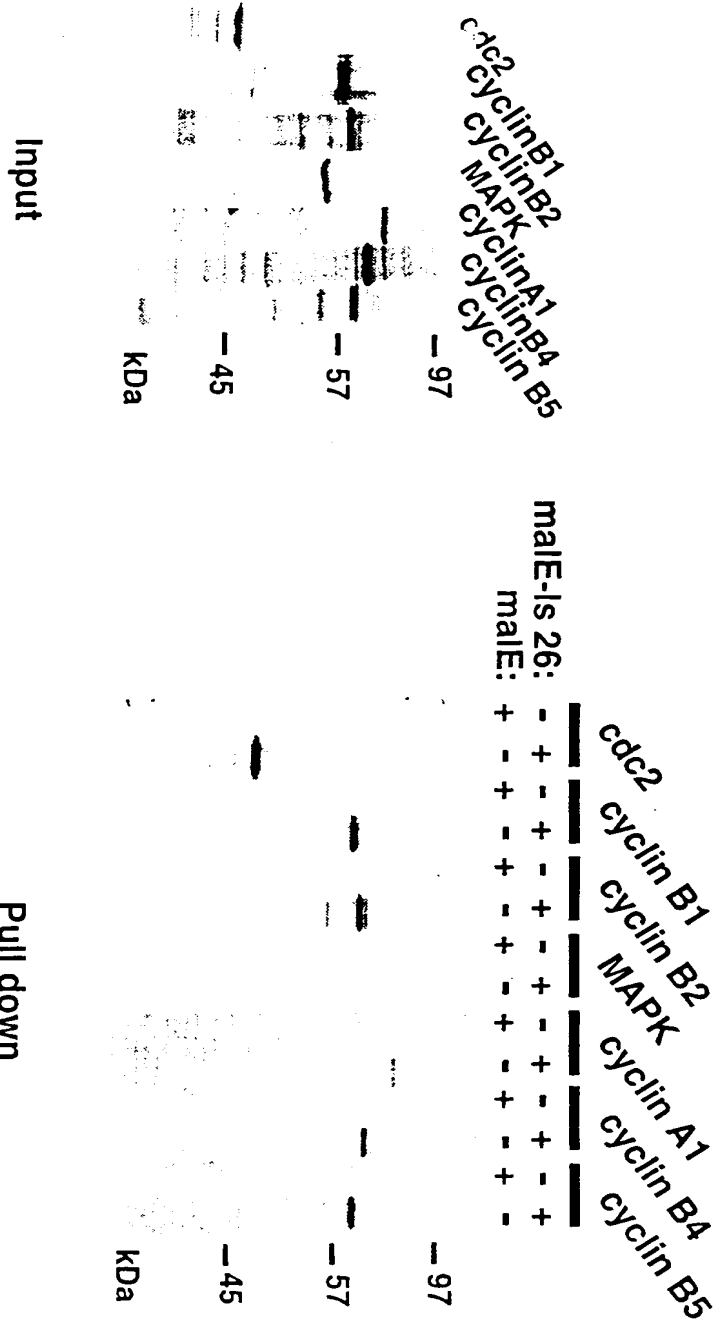


FIGURE 5



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Abstract

03. Feb. 1999

5 A DNA sequence according to the invention contains (a) a sequence as
shown in SEQ ID NO.1 or 2, (b) a sequence which encodes the same
protein as (a) but is degenerate as a result of the genetic code, (c) a
sequence hybridizing under stringent conditions to the sequences of (a)
and/or (b), (d) a genomic sequence containing the sequence of (a), (b) or (c)
and further containing one or more introns, or (e) a sequence which differs
10 from (a), (b), (c) or (d) due to its origin from a different species. A protein
according to the invention is encoded by such DNA sequence and can be
used for inducing oocyte maturation and/or promoting cell division and/or
differentiation and/or proliferation, in a pharmaceutical composition or as
diagnostic agent.

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